Methimzole and thyroid hormone replacement in broilers[☆]

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Abstract

Seven-day-old chickens were fed diets containing 18% crude protein $+ 0$ or 1 g methimazole/kg to produce either euthyroid or hypothyroid groups of birds at 28 days of age. These two groups were then offered diets containing either 0 or 1 mg triiodothyronine (T_3)/kg diet. Birds were sampled at 0, 2, 5, and 8 days following the onset of the T_3 treatment. Measurements taken at these intervals included in vitro hepatic lipogenesis (IVL), growth and feed consumption, hepatic enzyme activities (malic enzyme, ME; isocitrate dehydrogenase, ICD; and aspartate amino transferase, AAT), plasma hormones (T_3; thyroxine, T_4; insulin like growth factors I, IGF-I; and insulin like growth factors II, IGF-II) and metabolites (glucose; fatty acids, NEFA; triglyerides; uric acid). Hypothyroidism decreased IVL and ME at 28 days of age; however, T_3 supplementation for 2 days restored both IVL and ME. Paradoxically, continuing T_3 replenishment for an additional 3–6 days decreased IVL without affecting ME activity. In contrast, supplemental T_3 decreased IVL in euthyroid birds, regardless of the dosing interval, but had no effect on ME activity. Methimazole decreased plasma T_3, T_4, uric acid, and IGF-I, but did not affect IGF-II at 28 days. Giving T_3 to birds previously on methimazole increased plasma IGF-I as did feeding a control diet. Supplemental T_3 increased NEFA in both euthyroid and hypothyroid birds, but only for a short period following the initiation of supplementation (2 days post-supplementation). These data may help to explain some of the apparent reported dichotomies in lipid metabolism elicited by changes in the thyroid state of animals. In addition, most metabolic changes in response to feeding T_3 occurred within 2–5 days, suggesting that changes in intermediary metabolism preceded morphological changes. In conclusion, the thyroid state of the animal will determine responses to exogenous T_3.
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1. Introduction

Although the thyroid gland partially controls avian growth, artificial changes in thyroid hormone levels do not always change growth predictably. In one study [1], dietary triiodothyronine (T_3) and thyroxine (T_4) decreased body weight and feed efficiency of chickens. In a previous study [2], daily injections of thyroid releasing hormone (TRH) improved growth and increased plasma thyroid hormone concentrations. In contrast, feeding T_3 increased plasma T_3, but failed to improve the growth weight of dwarf chickens [3]. Cogburn et al. [4] also reported that dietary TRH increased plasma growth hormone (GH), thyroid hormone levels and body weight. On the other hand, long-term, dietary administration of thyroid hormones in another study decreased both growth and fat deposition, with T_3 being more effective than T_4 [5]. Other sets of data also suggested that dietary T_3 decreased body fat [4] as well as plasma GH concentrations [6]. It should also be noted that chemical hypothyroidism, caused by either propylthiouracil (PTU) or methimazole, also decreased growth [1,7].

What is lacking from these reports is any information concerning birds' recovery from thyroid hormone perturbations (inhibition of T_3 production). We proposed and tested the hypothesis that feeding T_3 after a 3-week methimazole challenge would rapidly restore circulating levels of T_3. Methimazole (1-methyl-2-mercaptimidazole), used to induce hypothyroid status in the present experiments, inhibits thyroidal production of thyroid hormones, but does not directly affect extrathyroidal 5'-deiodination of T_4 [8].

Malic (ME) enzyme activity was monitored because of its role in providing reducing equivalents (NADPH) for the synthesis of fatty acids. Isocitrate: NADP + oxidoreductase-[decarboxylating] (ICD) may function as both a residual source for the provision of NADPH and to provide a coreactant for transamination. Aspartate aminotransferase (AAT) aids in the removal of excess amine groups formed by feeding high-protein diets.

2. Materials and methods

2.1. Animals and diets

All chickens were held under a quarantine that was certified by the station veterinarian. Chickens were observed daily for healthiness. One authorized animal caretaker was assigned to maintain chickens over the course of the experiments. In addition, the research protocols were approved by the Beltsville Agricultural Research Animal Care Committee.

At 7 days of age, male, broiler chickens were assigned to one of two dietary treatments (180 g crude protein + 0 (C_1) or 1 g methimazole (M)/kg diet) for a 7–28-day growth trial. At 28 days, the chickens were given a diet containing 180 g crude protein + 0 (C_2) or 1 mg T_3 (T)/kg to result in four treatment groups (C_1–C_2, C_1–T, M–C_2, M–T). The first letter denoted the treatment from 7 to 28 days and the second letter the treatment from 28 to 36 days. These dietary treatments formed a factorial arrangement with four pen replicates for each dietary treatment. The experiment was replicated twice and 192 birds were sampled. The chickens were housed in battery-brooders in an environmentally controlled room maintained at 20–23 °C with a 12 h/12 h light/dark cycle (06:00–18:00 h

light). Chickens were selected at 08:00 h to minimize diurnal variation. One chicken was then randomly selected from each pen replicate at 28, 30, 33, and 36 days (a total of eight replicates for each treatment at every time increment), weighed, and bled by cardiac puncture into combination syringe-collection tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (Sarstedt Corp., Princeton, NJ, USA). The chickens were then killed by decapitation and the livers were rapidly removed into a PBS buffer and washed to remove blood and debris. The blood samples were centrifuged at $600 \times g$ and plasma samples were collected with individual Pasteur pipettes. Plasma samples were stored at $-80\,^\circ$C for later analyses of hormones and metabolites.

2.2. In vitro metabolism—lipogenesis

Livers were sliced (MacIlwain Tissue Chopper; 0.4–0.5 mm) to produce explants which were incubated at $37\,^\circ$C for 2 h in Hanks' balanced salts containing [9], 10 mM HEPES and 10 mM sodium[2-^{14}C]acetate (166 MBq/mol). All incubations were conducted in 3-ml volumes at $37\,^\circ$C for 2 h under a 95% O$_2$–5% CO$_2$ atmosphere [10,11]. At the end of the stated incubation periods, the explants were placed in 10 ml of 2:1 chloroform:methanol for 18 h according to Folch et al. [12]. The extracts were evaporated to dryness and dispersed in scintillation fluid. Radioactivity in the extracts was measured by liquid scintillation spectroscopy. In vitro lipogenesis was expressed as micromoles of acetate incorporated into lipids per kilogram body weight.

2.3. In vitro metabolism—enzyme assays

Remaining liver tissues were homogenized (1:10, w/v) in 100 mM HEPES (pH 7.5), 3.3 mM β-mercaptoethanol and centrifuged at $12,000 \times g$ for 30 min [10]. The supernatant fractions were kept at $-80\,^\circ$C until analyzed for the activities of malate: NADP + oxidoreductase-[decarboxylating] (ME, EC 1.1.1.40), isocitrate: NADP + oxidoreductase-[decarboxylating] (ICD-NADP, EC 1.1.1.42), and aspartate aminotransferase (AAT, EC 2.6.1.1). The activity of ME was monitored because of the enzyme's role in providing reducing equivalents (NADPH) for the synthesis of fatty acids. Isocitrate: NADP + oxidoreductase-[decarboxylating] may function as both a residual source of NADPH and to provide a coreactant for transamination. Aspartate aminotransferase aids in the removal of excess amine groups formed by feeding high-protein diets or during conditions of protein degradation.

Malic enzyme activity was determined by a modification of the method of Hsu and Lardy [13]. Reactions contained 50 mM HEPES (pH 7.5), 1 mM NADP, 10 mM MgCl$_2$, and the substrate 2.2 mM L-malate (disodium salt) in a total volume of 1 ml. Portions (50 μl) of the $12,000 \times g$ supernatants (diluted, 1:10) were pre-incubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at $30\,^\circ$C.

Isocitrate: NADP + oxidoreductase-[decarboxylating] activity was determined by a modification of the method of Cleland et al. [14]. Reactions contained 50 mM HEPES (pH 7.5), 1 mM NADP, 10 mM MgCl$_2$, and the substrate 4.4 mM DL-isocitrate in a total volume of 1 ml. Portions (50 μl) of the $12,000 \times g$ supernatants (diluted, 1:10) were pre-incubated in

the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 °C.

Aspartate aminotransferase activity was determined by a modification of the method of Martin and Herbein [15]. Reactions contained 50 mM HEPES, 200 mM L-aspartate, 0.2 mM NADH, 1000 U/l malate: NAD + oxidoreductase (EC 1.1.1.37) and the substrate, 15 mM 2-oxoglutarate in a total volume of 1 ml. Portions (25 μl) of the $12,000 \times g$ supernatants (diluted, 1:20) were pre-incubated in the presence of the first four ingredients. Reactions were initiated by adding the substrate and following the rate of oxidation of NADH at 340 nm at 30 °C. Enzyme activities are expressed as micromoles of product formed per minute under the assay conditions [10].

2.4. Plasma hormone assays

Both T_3 and T_4 concentrations were estimated with commercially available solid-phase single antibody kits (ImmuChem^{TM} Triiodothyronine and ImmuChem^{TM} Thyroxine, ICN Biomedicals, Irvine, CA). These assays were validated for avian samples [16] by dispersing standards in charcoal-stripped chicken serums and by noting recovery of added T_3 and T_4 (98%). Plasma insulin-like growth factors I and II (IGF-I and -II) were estimated with radioimmunoassays as previously described [17,18].

2.5. Plasma metabolite assays

Plasma triglycerides, uric acid, and non-esterified free fatty acids (NEFA) were determined with commercially available kits (Sigma Chemical Bulletin No's. 334-UV and 292-UV, Sigma Chemical Co., St. Louis, MO; NEFA-C, Wako Pure Chemical Industries LTD, Osaka, Japan). Each hormone or metabolite was measured in a single assay to remove inter-assay variation.

2.6. Statistical procedures

Significance of the two treatments (± methimazole) at 28 days was determined with a *T*-test. From this time on, the experiment was considered as a $2 \times 2 \times 3$ factorial arrangement. The main treatments were pre- and post-28-day thyroid status and time following initiation of the T_3 supplementation [19].

3. Results

Table 1 summarizes the effects of methimazole on in vitro lipogenesis and some hepatic enzymes whose activities parallel changes in lipogenesis. Feeding methimazole for 21 days depressed in vitro lipogenesis, malic enzyme and isocitrate dehydrogenase activities but did not affect aspartate aminotransferase activity. This table also summarizes the effect of methimazole on subsequent responses to supplemental T_3. Supplemental T_3, fed to birds given the control diet from 7 to 28 days (C_1–T), decreased in vitro lipogenesis 2 days following the onset of the T_3 supplementation. In contrast, 2 days of T_3 supplementation

Table 1
Effects of prior thyroid status and triiodothyronine (T_3) supplementation on in vitro metabolism[a,b]

	In vitro lipogenesis				Malic enzyme				Isocitrate dehydrogenase				Aspartate aminotransferase			
	Day				Day				Day				Day			
	0	2	5	8	0	2	5	8	0	2	5	8	0	2	5	8
C_1–C_2	40.1	35.3	35.1	32.7	23.6	23.8	20.8	16.5	33.8	34.9	47.5	28.4	56.5	52.5	43.8	47.6
C_1–T		22.3[d]	19.5[d]	12.6[d]		21.2	19.6	22.6		32.4	34.4	30.7		57.8	67.3	48.7
M–C_2	15.5[c]	14.5	35.9[d]	27.1[d]	3.2[c]	3.9	14.2[d]	19.7[d]	27.9	27.5	31.7	28.6	52.9	55.5	67.3	55.2
M–T		33.9[d]	20.9[d]	22.3[d]		12.1[d]	16.8[d]	21.2[d]		41.9[d]	34.9	27.8		98.8[d]	68.7	51.1
Pooled SE	2.72				6.43				5.55				13.07			

[a] The first letter in each legend denotes the dietary treatment for the 7–28-day period (C_1 = control diet containing 18% protein; M = control + 1 g methimazole/kg diet). The second letter denotes the treatment for the 28–36-day period (C_2 = control diet; T = control + 1 mg T_3/kg diet). Birds were sampled at 0, 2, 5, and 8 days following the initiation of T_3 supplementation.

[b] In vitro lipogenesis is expressed as micromoles of [2-^{14}C]acetate incorporated into per gram hepatic lipids per 2-h incubation; malic enzyme, isocitrate dehydrogenase, and aspartate aminotransferase are expressed as enzyme units per gram of liver.

[c] Significant ($P < 0.05$) effect of methimazole at 28 days.

[d] Significant difference between a 28-day value and a treatment on a particular day.

Table 2
Effects of prior thyroid status and triiodothyronine (T_3) supplementation on plasma hormone concentrations[a,b]

	IGF-I				IGF-II				T_3				T_4			
	Day				Day				Day				Day			
	0	2	5	8	0	2	5	8	0	2	5	8	0	2	5	8
C_1–C_2	4.55	5.8	5.1	5.5	1.50	1.7	2.3	2.4	4.7	3.8	3.1	3.2	21.9	19.0	14.1	16.8
C_1–T		5.0	5.1	4.9		1.6	1.8	2.4		12.8	12.4	13.0		6.1[d]	4.9[d]	5.2[d]
M–C_2	0.41[c]	1.2	2.9	3.4	1.62	2.1	2.2	2.4	1.8[c]	6.3[d]	6.5[d]	4.9[d]	7.2[c]	8.2	9.0[d]	10.2[d]
M–T		2.3	3.4	3.8		2.1	2.5	2.2		14.4[d]	13.2[d]	15.0[d]		5.7	5.1	4.7
Pooled SE		0.08				0.08				0.58				0.82		

[a] For an explanation of the treatment legends, see Table 1.
[b] T_4, thyroxine; IGF-I, insulin-like growth factor-I; IGF-II, insulin-like growth factor-II. Hormone concentrations are expressed as nanomoles per liter.
[c] Significant ($P < 0.05$) effect of methimazole at 28 days.
[d] Significant difference between a 28-day value and a treatment on a particular day.

Table 3
Effects of prior thyroid status and triiodothyronine (T_3) supplementation on plasma metabolite concentrations[a,b]

	Glucose				NEFA				Uric acid				Triglycerides			
	Day				Day				Day				Day			
	0	2	5	8	0	2	5	8	0	2	5	8	0	2	5	8
C_1-C_2	275	282	276	265	210	204	182	165	6.7	6.9	6.4	7.0	126	108	137	102
C_1–T		253	263	244		694[d]	355[d]	337[d]		4.2[d]	4.2[d]	4.8[d]		77[d]	90[d]	73[d]
M–C_2	319	296	252[d]	259	209	184	240	263	9.3[c]	9.5	6.2[d]	3.9[d]	129	121	108	90[d]
M–T		238	262	254[d]		697[d]	300[d]	255		4.3[d]	2.9[d]	4.1[d]		83[d]	69[d]	76[d]
Pooled SE			6.5				12.1				0.17				8.1	

[a] For an explanation of the treatment legends, see Table 1.
[b] Plasma NEFA, plasma non-esterified fatty acids.
[c] Significant ($P < 0.05$) effect of methimazole at 28 days.
[d] Significant difference between a 28-day value and a treatment on a particular day.

increased lipogenesis in birds previously given methimazole (M–T). In contrast, after 5 days of supplementation, T_3 decreased lipogenesis in this same group. Treatment effects on ME and ICD activities were somewhat less definitive. For example, T_3 supplementation had no effect on ME or ICD activities euthyroid birds. In contrast, T_3 increased ME activity in hypthyroid birds 2 days following the initiation of supplementation, but had no further effect as the supplementation period lengthened. Furthermore, ME activity in this group (M–T) was equal to that group switched from methimazole to a control diet (M–C_2) at 8 days. It was evident that supplemental T_3 merely accelerated the adaptation in ME activity.

Table 2 summarizes the effects of both methimazole and supplemental T_3 on plasma hormone concentrations. As expected, methimazole decreased plasma T_3 and T_4 at 28 days. Methimazole also decreased plasma IGF-I without changing plasma IGF-II. Giving T_3 to birds previously on methimazole (M–T) increased plasma IGF-I as did feeding a control diet (M–C_2). Although exogenous T_3 restored IGF-I values at a much faster rate, 8-day values were similar in both groups. Likewise, exogenous T_3 rapidly restored plasma T_3 and neutralized the effect of the prior thyroid state. In contrast, exogenous T_3 decreased plasma T_4, regardless of the prior thyroid state. The present study demonstrated that a hypothyroid state would depress plasma IGF-I compared to the euthyroid state. Furthermore, normalization of the thyroid status resulted in a partial restoration of IGF-I values.

Table 3 summarizes the effects of methimazole on certain plasma metabolites. Methimazole increased ($P < 0.05$) plasma uric acid levels, but had no effect on plasma glucose, NEFA or triglycerides. Supplemental dietary T_3 increased ($P < 0.05$) NEFA 2 days following the onset of supplementation. It should be noted that background thyroid status had no effect on this response (C_1–T versus M–T). Supplemental T_3 also lowered ($P < 0.05$) plasma uric acid levels in birds previously treated with methimazole (M–T). It should be noted that merely switching the birds from a diet continuing methimazole to a control diet (M–C_2) also lowered plasma uric acid, but not as rapidly as M–T.

4. Discussion

It was of interest to note that T_3 supplementation of euthyroid birds had no effect on IGF-I status. Vasilatos-Younken and Scanes [20] have previously discussed the possibility that patterns of growth secretion may regulate plasma IGF-I in chickens. We have previously shown that supplemental T_3 severely depressed plasma GH and have speculated that this depression might affect plasma IGF-I levels [21]. Although, depressed IGF-I values in humans can be returned to normal with T_4 replacement [22,23], it was somewhat surprising that T_3 had no effect upon plasma IGF-I concentrations in the present study after 8 days of supplemental T_3.

Decuypere et al. [24] mentioned that methimazole depressed growth and increased fatness, but that administration of thyroid hormones decreased both growth and fat deposition. They attributed reduced growth of methimazole-treated birds not to the hypothyroid state but to decreased IGF-I production. The present study agrees with these results and extends knowledge by implying that LPL may cause the increase in fatness seen in the hypothyroid birds.

Serum concentrations of GH in birds were not affected by propylthiouracil (PTU) or T_4 treatments, whereas serum IGF-I levels were significantly decreased in PTU-treated chickens [25]. The lowered serum IGF-I levels in the PTU-treated group were completely restored to the control levels by T_4 injections. Previous work with PTU-fed cockerels demonstrated that serum levels of GH, but not IGF-1, tended to rise and those of IGF-binding activity to fall. Thyroxine supplementation to PTU-fed cockerels for 8 days induced hyperthyroidism and reversed these serum parameters. This work also suggested that T_3 increases unsaturated IGF-binding proteins by reducing circulating IGF-1 concentrations [26].

It could be speculated that the large decrease in NEFA after day 2 of supplementation was a result of a loss in readily mobilized depot fat. This hypothesis seems to be supported by a rapid drop in NEFA after this transient elevation. Furthermore, the initial increase in NEFA did not relate to the background thyroid status of the birds (euthyroid versus hypothyroid). In contrast, reverse triiodothyronine (rT_3) antagonizes the hypermetabolic effect of T_3. For example, previous experiments revealed that exogenous rT_3 increased NEFA in pullets and adult chickens [27]. It appears that the lipolytic effect of T_3 is, indeed stereospecific as demonstrated by the antilipolytic effect of rT_3. The noted decrease in plasma triglycerides following T_3 supplementation (seen in both euthyroid and hypothyroid birds) seems interesting in light of the increase in lipogenesis seen in hypothyroid birds given T_3 (2 days post-supplementation). It is possible that T_3 mobilizes peripheral fat stores to such a degree that plasma triglycerides drop in response to a need to replenish these peripheral stores. In any case, it may be difficult to determine the dynamics of plasma triglycerides from a single point value. To a certain degree, interpretation of single point uric acid values may be as problematic as triglycerides although it appears that T_3 may stabilize protein degradation (depress plasma uric acid) while hypthyroidism has the opposite effect.

The data strongly indicate that restoration of normal levels of T_3 is necessary to maintain lipid metabolism in the 29-day-old broiler. Up to this time, we have reported that supplemental T_3 only decreased lipogenesis in the euthyroid broiler. The present study shows that supplemental T_3 enhanced lipogenesis in the hypothyroid chicken, but that enhancement only lasted until restoration of normal levels of plasma T_3 occurred. At this point, it should be noted that T_3 supplementation did not restore lipogenesis to control levels although plasma T_3 concentrations were much greater than controls. Nonetheless, restoration of plasma T_3 is important because T_3 regulates both the metabolism of chickens and the flux of calories supporting metabolism. In addition, T_3 may alter the sensitivity of an organ to other regulatory hormones and tissue factors as well as directly influence the metabolic rate of that organ [28].

Although the data in the present study indicate that T_3 depresses lipogenesis in the euthyroid bird, it has little effect on the activities of certain lipogenic enzymes. In this respect, our data provide a contrast to other work. For example, Clarke and Hembree [29] found an increase in malic enzyme activity in rats injected with T_3. Previous articles describing metabolism in rodents work also showed that T_3 increased lipogenesis [30] and lipogenic enzyme activities [31]. Early analyses indicate that ME activity may support a relationship between T_3 level and hormone action at the cellular level [32]. Thus, a depression in either T_3 binding or circulating levels (as is the case in the present study) would result in decreased enzyme activity and subsequent de novo lipogenesis.

Triiodothyronine administration has been reported to increase rat liver malic enzyme mRNA abundance by possibly altering transcriptional events, nuclear processing or mRNA turnover [33]. Triiodothyronine stimulates transcription by forming a nuclear hormone-receptor complex that enhances transcription [34]. It should follow that a relative increase in mRNA abundance results in an increase in enzyme protein synthesis and an increase in activity [29]. It is also important to realize that although ME may provide the necessary NADPH for lipogenesis, the enzyme may not strictly regulate lipogenesis according to the data in the present study. A more plausible explanation is that ME reflects NADPH utilization and may not regulate fatty acid synthesis.

The findings of this study indicate a close relationship between thyroid status, fat metabolism and subsequent fat deposition and may help to alleviate some of the conflicts concerning the role of the thyroid in lipid metabolism. Although our previous findings demonstrate unequivocally that exogenous T_3 depresses de novo lipogenesis, it can also be shown that normal levels of endogenous T_3 are required for this effect [35]. A recent study [36] showed that T_3 stimulates a seven-fold increase in transcription of the acetylCoA carboxylase-alpha (ACC-alpha) gene in chick embryo hepatocytes. These data suggest that T_3 regulates ACC-alpha transcription by changing the composition of nuclear receptor complexes bound to a ACC-alpha–T_3 response element. At this time, it is important to note that ACC catalyzes a proposed rate limiting step in de novo lipid synthesis and that T_3 may be necessary for transcription of its gene.

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